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Subclinical porcine circovirus infection significantly decreases growth parameters of fattening pigs

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1. Abstracts

1.1. English

Porcine Circovirus Type 2 (PCV2) is the obligate infectious agent in Postweaning Multisystemic Wasting Syndrome (PMWS) of pigs. We vaccinated dams twice before and once during pregnancy with an inactivated PCV2 vaccine and monitored health status, antibody titers in dams and piglets and growth parameters of their progeny. Two producer farms, run under the supervision of the Swiss Swine Health Organisation and categorized in the highest health status, were selected for the study. Historically, PMWS was diagnosed in animals of one farm at the weaning stage, whereas in the other farm, pigs wasted during the fattening period. Groups of dams in each farm were randomly chosen for vaccination or sham injection.

Compared to historical data, cases of PMWS decreased 4-12-fold among all animals of both farms. In sera of vaccinated dams only low concentration of PCV2 DNA was detected and no case of PMWS was diagnosed in their progeny. Vaccination increased serum antibodies of dams significantly accompanied with significant increased colostral antibody titers in their offspring. However, after weaning, progeny from vaccinated dams showed a significant increased daily weight gain and shortened fattening period compared to controls. This is the first demonstration of an effect of subclinical circovirus infection on growth performance of fattening pigs that can be controlled by vaccination of dams.

1.2. German

Porcine Circovirus Typ 2 ist der notwendige Erreger beim Multisystemic Wasting Syndrom (PMWS) beim Schwein. In unserem Versuch wurden Mutterschweine zweimal mit inaktiviertem PCV2 Impfstoff vor und einmal während der Trächtigkeit geimpft. Von Muttersauen und Nachkommen wurden Daten zum Gesundheitsstatus, Antikörperverlauf und Mastleistungen erhoben. Für den Feldversuch wurden zwei SDG Zuchtbetriebe mit dem höchsten Gesundheitszustand ausgewählt. Während PMWS beim einen Betrieb früher während der Absetzphase aufgetreten ist, traten beim anderen Betrieb Probleme mit PMWS erst während der Mastperiode auf. Im Versuch wurden die Muttersauen in beiden Betrieben zufällig in eine Impf- bzw. Placebogruppe eingeteilt. Im Vergleich zu früher haben die PMWS-Fälle auf beiden Betrieben um das 4-12fache abgenommen. In Seren von geimpften Muttertieren konnte PCV2-DNA nur niedriger Menge nachgewiesen werden. PMWS Fälle traten nur noch bei den Nachkommen von ungeimpften Kontrollsauen auf. Die Impfung gegen PCV2 erzeugte eine signifikante Erhöhung der Serumantikörpertiter bei den Muttertieren bzw. der Kolostralantikörpertiter bei deren Ferkel. Im Vergleich zur Kontrollgruppe wiesen Nachkommen geimpfter Muttertiere eine erhöhte Masttageszunahme und eine verkürzte Mastdauer auf. Erstmals konnte die Wirkung einer Mutterschutzimpfung auf das Wachstum von Mastschweinen in einem subklinisch mit PCV2 infizierten Betrieb gezeigt werden.

2. Introduction

PMWS in pigs was first described in Canada [1] and has since been recognized as one of the economically most important swine diseases worldwide [2-8]. PMWS emerged as an epizootic disease in Switzerland in 2003/2004 even though most pig farms were kept under specific pathogen free animal conditions monitored by the Swiss Swine Health Organisation [9].

PMWS is an acute or chronic disease in animals around the age of 5-16 weeks [10, 11], or exceptionally to 30 weeks [12]. Typical signs are wasting, profuse diarrhoea, dyspnoea and pigs may have gastric ulcers, enlarged lymph nodes, anaemia, icterus, haemorrhages, dermatitis, nephropathy, vasculitis or edema in various organs [1, 11, 13-15].

Various PCV2 genotypes are considered to be the main aetiological agent of PMWS [3-7, 14, 16]. Nevertheless, PCV2 can be detected in healthy pigs or isolated from various cells and organs including peripheral blood, mononuclear cells, dendritic cells, lymphocytes, and viral antigen is often found in defined lymphatic areas in lymph nodes, tonsils, spleen, and thymus or is scattered in their supporting reticular cells associated with irregular tissue architecture and in macrophages [14, 17]. In other cases, PCV2 was diagnosed in lung, liver, kidney and gastro intestinal tract and in rare cases in apoptotic vascular endothelial cells of the brain [18].

As PCV2 can replicate in multiple cells of various organs to measurable titers in clinically healthy or diseased animals, the virus may be present in sera or all other body fluids [11, 13] including semen [19, 20]. Infection of naïve animals may occur by direct contact with infected animals, their secretions or air borne dissemination is considered due to high

viral loads in large farms [21]. In addition, natural vertical transmission was diagnosed in field cases [22, 23] or could be induced experimentally [24, 25]. Experimentally infected dams delivered dead and stillborn piglets. PCV2 infection in fetuses was verified and was associated with myocarditis, fibrosis and degeneration of the myocardium as well as depletion of lymphocytes [26, 27]. Recent evidence further suggests that intrauterine infection may have been underestimated at least in some herds [28].

In a retrospective epidemiological study PCV2 could be traced back to 1979 in Switzerland [9]. Nevertheless, it was not until 2001 when the first clinical PMWS case was confirmed [29]. However, the epizooty started in the late 2003 in areas with high swine populations [30].

Since, PCV2 is endemic and can be isolated from PMWS diseased and clinically healthy animals. PCV2-specific antibodies are detected in almost all pigs [11, 31-35]. Another complicating factor is the observation that the profiles of PCV2 serum antibody titers of pigs from PMWS affected and unaffected herds are almost identically [36, 37]. Thus, the presence of PCV2 specific IgG antibodies are of limited diagnostic or prognostic value and should be considered for diagnostics in conjunction with disease pattern and PCV2 viral load [38].

Until recently, the main focus in reducing PMWS was the optimization of herd management in general [39, 40] and intensifying the survey of health program such as those run by the Swiss Swine Health Organisation (www.suisag.ch/SGD/Richtlinien). Despite the absence of Porcine Reproductive and Respiratory Syndrome (PRRS), Enzootic Pneumonia (EP), Actinobazillosis (APP), progressive Atrophic Rhinitis (pRA) in Switzerland, PMWS occurred in such herds [9]. Hence, other measures had to be taken to control the disease. Vaccination against PCV2 was thus considered.

Two types of vaccines against PCV2 were introduced in Europe. One is used to vaccinate pregnant sows to increase colostral antibody concentration and the other is used to vaccinate piglets. Several field studies have demonstrated that vaccination of nursing piglets is effective in reducing losses caused by PMWS and that maternal antibodies present at the time of vaccination did not interfere with active antibody production [41-43].

In the present study, pregnant sows from two different farms under the control of the Swiss Swine Health Organisation with a PMWS history were immunized. The ubiquitous presence of PCV2 may facilitate intrauterine or perinatal infection [44]. Vaccination of pregnant dams against PCV2 may decrease overall viral load perinatally and increased colostral antibodies may protect offspring within the first day of life. To test the effectiveness of vaccination reproductive parameters, antibody production of the dams and antibody transfer to piglets, mortality rate, growth performance of offspring and age of slaughter were analyzed.

3. Materials and Methods

3.1 History of the herds

This study took 15 months. We used Circovac® (Merial SA, Lyon) in two different farms with a history of recurrent PMWS. In both herds PMWS has been diagnosed before the start of the study using criteria defined by Sorden [38] and used as described by Wiederkehr et al [9].

Both herds were on a health program run by the Swiss Swine Health Organisation and were categorised in the highest health category and declared free of PMWS cofactors as

Porcine Reproductive and Respiratory Syndrome (PRRS), Enzootic Pneumonia (EP), Actinobazillosis (APP), progressive Atrophic Rhinitis (pRA) and mange. In both farms the piglet were weaned at the age of 4-5 weeks of life.

Herd X was a breeding herd with 90 Swiss Large White sows. Gilts were raised for replacements within the farm. Piglets were sold to a regional finisher at the age of approximately 10 weeks at a body weight of 22-27 kg. Only sporadic cases of PMWS had occurred in herd X since 2006, but there had been considerable losses in the finishing operations. 10-15% of these pigs developed PMWS as verified by pathological examination.

Herd Y was a breeding herd with 150 Swiss Large White x Landrace crossbred sows. Gilts were purchased from another farm and added to this herd without quarantine. 1-2 weeks after weaning approximately 5-20% of the weaned pigs had low daily weight gain and profuse untreatable diarrhoea with a herd mortality rate of 5-10%, indicators of a serious PMWS problem confirmed by laboratory diagnostics.

3.2. Vaccination protocol

Sows were randomly chosen for either vaccination or were injected with adjuvant as a control group. The inactivated PCV2 vaccine Circovac® (Merial SA, Lyon) was used at a dose of 2 ml administered deep into the neck musculature, using a 1.2x40 mm needle, four and two weeks before artificial insemination and four weeks ante partum. One 2 ml-dose contained ≥2.1 $\log_{10}$ PCV2 antigenic units, 0.2 mg thiomersal and 500 mg paraffin as an adjuvant.

Furthermore for some comparisons, the dams were categorised as young (≤3 litters, n=65) or experienced dams (>3 litters, n=159) as indicated in the data sets.

3.3 Blood collection for serum production

10 ml blood was collected from the jugular vein of dams immediately before the first injection (B0), four weeks after the second injection (B1) and two weeks after the third injection (B2). 2-5 ml blood was collected from 100 individually tagged piglets at the age of 3, 10, 31, 42, 56, 63 days post partum (pp). This study was carried out according Swiss Animal Welfare guidelines (study number 06/07).

3.4 Serological examinations

A competitive ELISA (SERELISA® PCV2 Ab Mono Blocking Systems (Synbiotics Corporation Europe SAS, Lyon) was used for antibody (IgG) detection [45]. The completion of the test, data analysis and transformation of the data into ELISA units were done according to the manufacturer's instructions and a published reference [45]. We supplemented the assay with two additional controls to the sera dilutions suggested by the manufacturer. Firstly, we used an additional positive and negative control serum to check plate antigen coating homogeneity. Secondly, we normalized S-values among individual plates with the aid of a known serum.

Immunoglobulin M (IgM) was measured using INGEZIM CIRCOVIRUS IgG/IgM (Ingenasa, Madrid) a capture immunoenzymatic assay specific IgM antibody detection to PCV2.

3.5 Production variables of sows

Parity number, litter weight, number of live and dead born piglets, number of mummies, number of piglets weighing below 1 kg, the number of piglets lost during the nursing period and the cause of death were recorded for each dam.

3.6 Production variables of progeny

Cross-fostered piglets from large to small litters were not considered in this study. The variables Average Daily Weight Gain ($ADWG^1$) is generated as live slaughter weight (kg) divided per age in days. $ADWG^2$ is generated as live slaughter weight (kg) minus weight at the beginning of the finishing period (kg) divided per number of finishing days. The carcass weight was considered to represent 78% of the live weight at slaughter and was used to calculate the latter. The number of finishing days was calculated from the dates of slaughter and weaning.

3.7 Pathological examinations

All mummies, stillborn and dead nursing piglets as well as the dead weaning and fattening pigs, were examined at the Institute of Veterinary Pathology, Vetsuisse Faculty of Zurich. If deemed necessary, histological, bacteriological and virological examinations were also inducted. When PMWS was suspected, tissues were examined immunohistochemically (IHC) and with PCR for PCV2 infection [9, 46, 47].

3.8 Statistics

Statistical calculations were carried out using StatView 5.1 (SAS Corporation). ANOVA for repeated measures, unpaired and paired *t*-tests were considered statistically significant when $p \leq 0.05$ (*).

4. Results

4.1 Occurrence of PMWS during the vaccination period

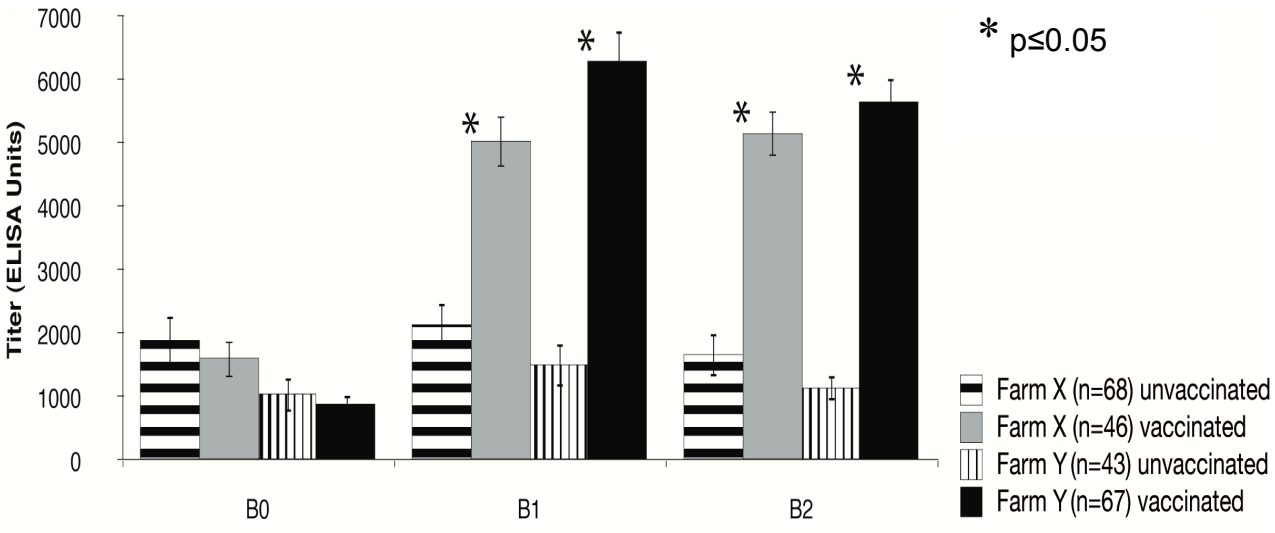
This study was conducted within a period of 15 month. During this time, 379 dead piglets of a total of 2720 (13.9%) born piglets occurred and underwent a post mortem examination. 176 were from herd X and 203 from herd Y. This number includes aborted, stillborn piglets and perinatal losses. The majority of the piglets were crushed by their mothers during the first week of life. There were no significant differences in the number of piglets lost between the vaccinated and non-vaccinated dams in both herds. 60 of the dead piglets were examined immunohistochemically for the presence of PCV2 antigen. All were negative.

At the weaning stage, 9 of 1169 weaned pigs died in herd X (0.8%) and 15 of 1172 in herd Y (1.3%). PMWS was diagnosed in none of these pigs.

During the fattening period a total of 423 pigs were monitored until slaughter (112 of herd X and 311 of herd Y). PMWS was diagnosed in 2 of the 112 pigs born to two non-vaccinated sows of herd X (1.8%). Interestingly, no case of PMWS was diagnosed in 311 fattening pigs from herd Y.

Compared to the historical data the mortality rate at the weaning stage of herd Y dropped from an average of 8.7% to 1.3% and in the finisher of herd X from 10-15 % to 1.8%. Vaccination may have had an effect on the overall health status of vaccinated and control animals by reducing viral pressure in general and in the antibody production in particular. Next, we analyzed the development of antibodies in dams and their offspring.

Figure 1. Antibody titers of vaccinated and unvaccinated dams.



At the start of the experiment (time B0), 99% of all dams had antibodies against PCV2. In herd X, the mean titer at B0 of all sows was significantly (p=0.006) higher than that of herd Y (Fig. 1; Table 1). At time points B1 and B2, vaccinated dams of both herds had significantly higher titers against PCV2 than unvaccinated dams. In the vaccinated ones there were significant differences in serum titers between B0 and B1 in both herds. Titers increased up to nine-fold compared to the titres at B0.

We noted a fluctuation in titers over time in the unvaccinated dams that may have been due to subclinical infections. The dams of herd Y had a lower titre at time point B0 than herd X, (Table 1). The unvaccinated controls showed a mild but significant increase in titer (herd X, p=0.03; herd Y, p=0.04) between time points B0 and B1, but no significant difference between B1 and B2. Only low concentrations of PCV2 DNA (<$5x10^5$ Copies /ml) were detected by PCR in some sera (data not shown).

A closer analysis of titers between young and experienced dams revealed some interesting observations (Table 1). Young dams had a 3.5-fold higher baseline titer (B0) than experienced dams ($p<0.0001$). At B1, vaccinated young dams had the highest titer (6820 EU) measured during the entire study.

Table 1. Antibody titers of dams in Farm X and Y, young and experienced dams and piglets

A: Antibody titers (ELISA Units) of all dams in herd X and Y

	herd X			herd Y		
Time	vaccinated (n=46)	unvaccinated (n=68)	p-value	vaccinated (n=67)	unvaccinated (n=43)	p-value
B0	1613±306	1893±369	>0.05	887±157	1046±278	>0.05
B1	5035±410	2140±339	<0.05	6303±487	1508±345	<0.05
B2	5156±358	1670±344	<0.05	5661±358	1140±193	<0.05

B: Antibody titers in young and experienced sows

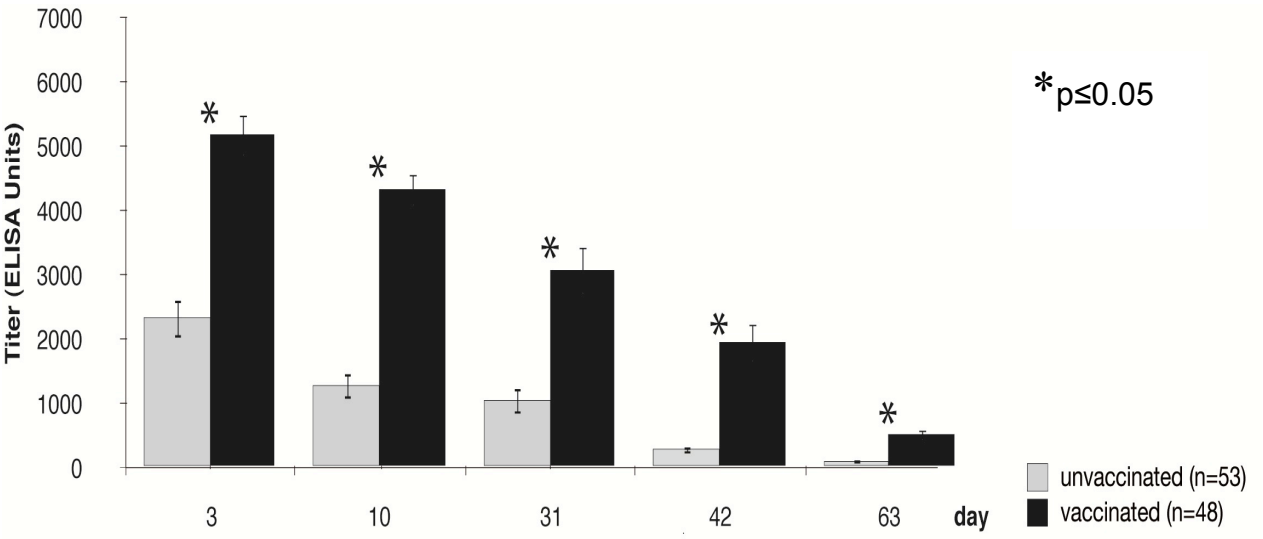
	young dams			experienced dams		
Time	vaccinated (n=42)	unvaccinated (n=24)	p-value	vaccinated (n=71)	unvaccinated (n=88)	p-value
B0	2229±781	2229±331	>0.05	874±173	564±105	>0.05
B1	6820±511	3255±929	<0.05	5176±431	1360±184	<0.05
B2	6404±475	2068±531	<0.05	4895±422	1157±193	<0.05

C: Antibody titers in piglets from vaccinated or unvaccinated dams of herd Y

Time pp.	vaccinated (n=53)	unvaccinated (n=48)	p-value
03	5173±322	2323±308	<0.05
10	4319±248	1266±203	<0.05
31	3062±359	1037±206	<0.05
42	1943±296	279±69	<0.05
63	511±81	86±31	<0.05

B0 (before 1th vaccination), B1 (four weeks after second injection), B2 (two weeks after third injection) with mean antibody titer and standard deviation.

Figure 2. Antibody titers in piglets



4.2 Antibody titers in piglets

For these experiments sera from 101 offspring of vaccinated and unvaccinated dams were collected in herd Y. At all times, the piglets from vaccinated dams had significantly higher PCV2 antibody titers than piglets from unvaccinated dams ($p<0.001$; Table I; Fig. 2). From day 3 post partum (pp) to 63 pp, the antibody titer in the vaccinated group decreased gradually from 5173 EU to 511 EU. In the piglets from the unvaccinated ones, the antibody level decreased from 2323 EU at day 3 pp to 86 EU at day 63 pp. The decrease was gradually until day 63 pp with a drop at day 31 pp. At no time point a significant seroconversion was noted as previously described [42]. This is paralleled by the absence of any case of PMWS at this stage.

To detect potential subclinical infections, the sera of all piglets from unvaccinated and vaccinated dams collected at day 10, 31, 42, 56 and 63 were examined for IgM antibody against PCV2. Four piglets from unvaccinated sows in herd Y were positive at day 56 indicating subclinical infections.

4.3 Offspring production parameters

Offspring of vaccinated dams from both herds X and Y had significantly greater daily weight gains from birth to slaughter compared with the controls of unvaccinated dams (Table 2). The difference of ADWG[1] between progeny from vaccinated and unvaccinated dams in herd X was 33 g/d and in herd Y 20 g/d. From weaning to slaughter the difference between offspring of vaccinated and unvaccinated dams was 51 g/d in farm X and 30 g/d in farm Y. The age at slaughter of pigs from the vaccinated ones was reduced in herd X by 6.7 days (p=0.03) and by 5.5 days in herd Y (p=0.02). Therefore, the increased antibody titer in dams, transmitted by colostrum was associated with a significant impact on health status of the offspring reflected by increased weight gain.

Table 2. Growth performance of progeny

Production parameters of offspring from vaccinated and unvaccinated dams in farm X and Y

	progeny farm X (n=112)			progeny farm Y (n=311)		
	vaccinated (n=57)	unvaccinated (n=55)	p-value	vaccinated (n=182)	unvaccinated (n=119)	p-value
ADWG[1] (g/d)	626±0.01	593±0.01	<0.05	585±0.01	565±0.01	<0.05
ADWG[2] (g/d)	785±0.02	734±0.02	<0.05	726±0.01	696±0.01	<0.05
Age of slaughter (d)	170±1.9	176±2.2	<0.05	183±1.8	189±1.4	<0.05

5. Discussion

Dam vaccination was associated with a stunning improvement of the health status of offspring at the weaning stage and especially of the fattening period. Moreover, offspring of vaccinated dams outperformed pigs from unvaccinated dams in weight gain and decreased time to reach maturity for slaughter. We associated these economic benefits with low PCV2 viral pressure, due to vaccination of dams and their transfer of colostral antibodies to progeny in particular.

Historically and during the experiment none of the two farms selected for our experiments had cases of reproductive disorders due to PCV2 according to the records of the Swiss Swine Health Organization (unpublished data). Furthermore, all of the sera collected from adult animals had low concentration of PCV2 DNA ($<5x10^5$ copies/ml). The perinatal loss of some 14% was not affected by vaccination and is in the range of small to mid-sized pig farms surveyed by the Swiss Swine Health Organization (Geschäftsbericht SUISAG; *Zahlen und Projekte* 2010). Therefore, the existing natural immune responses of the dams against PCV2 enhanced by vaccinating dams appeared sufficient to prevent fatal intrauterine infection.

During the experiment, we did not find any indication of PCV2 associated diseases in the 379 analyzed dead pigs. By contrast, the historical loss of 5-10% of pigs after weaning or in the early fattening period respectively was improved 6-12 fold during the experiment. Only 2 of the 423 evaluated fattening pigs (1.3%) died of PMWS during the experiment in the early finishing period. Interestingly, the two pigs were born to unvaccinated dams. Therefore we suggested that vaccination significantly improved the health status of all offspring irrespectively of the vaccination status of the dams and might indeed be due to an overall lowered infectious pressure of PCV2.

As expected from previous epidemiological and serological examinations all dams had antibodies from natural exposure to PCV2 before vaccination [21, 37]. In contrast to other reports [41, 48] we opted to vaccinate twice before and once during pregnancy to compensate for a potential loss of serum antibodies into the colostrum [49]. Vaccination boosted antibody titers 3-9 fold after one vaccination that leveled off or decreased after the second immunization. This phenomenon was observed in the unvaccinated dams, bled at the same time, as well.

The PCV2 specific antibody titer in the sera from piglets of vaccinated dams was very similar to those of their mothers as previously described [49]. The antibody titer in these piglets decreased gradually about 10 fold within 60 days and was in average 6 times higher than that of the controls at the same time point. Antibody titers against PVC2 in neonatal piglets of unvaccinated dams also reflected that of their mothers in the first few days of life [49], but the decrease of the mean titer in these animals until day 63 was 30 fold. The antibody titers of progeny from vaccinated dams was in the 6^{th} week of life about the same as the titer of the controls at third day of life. In addition, the decrease of the titer was not gradual but had a sharp drop after day 31. We suspected subclinical PCV2 infection in those pigs. Thus, we tested the presence of IgM antibodies isotype specific to PCV2 and attempted to PCR amplify viral DNA. Notably, four of 53 pigs of the unvaccinated group developed IgM antibodies at the age of 56 days pp against PCV2 but none of the vaccinated group. Nevertheless, we could not detect PCV2-DNA in blood of these piglets (data not shown). Based on the observed IgM antibodies we concluded that these four piglets were newly subclinically infected with PCV2 as antibodies of this isotype were not detected at earlier time points (data not shown).

The improvements in the general health status of pigs from vaccinated dams were further observed in the significant higher daily weight gain and decreased time to slaughter when compared to offspring from unvaccinated dams. It is tempting to speculate that the improved immune status of vaccinated dams and the increased colostral antibodies taken up by their offspring decreased the overall infectious pressure. Indeed, before the vaccination experiments the antibody titres of sows in both farms was generally lower.

Although a direct comparison of results in an industrial setting is difficult. Vaccination of dams, as done here, rather than 2-3-weeks old piglets [42, 50, 51] might have some advantages. Perinatal virus load of gilts or piglets may have been underestimated at least in some herds [28, 52]. PCV2 can infect a variety of cells and replicates preferentially in dividing cells [17]. Dividing cells are very important to reach adult stage cell numbers and are crucial for the development of the architecture of organs of the immune system, the gastro intestinal tract and the central nervous system. Failure or delayed maturation of the immune system may favour secondary infections or growth retardation due to inadequate food intake or inadequate hormonal regulation of body growth [53].

It is very difficult to induce a protective immune response right after birth in mammals. Therefore, piglets are generally vaccinated at three to four weeks of age. During this time the animals are protected by maternal antibodies that may vary depending on the immune status of the dam and thus, piglets will be exposed to the PCV2 viral pressure present in the herd with potentially devastating effects. The vaccination of dams appeared to decrease the infective pressure generally and provided increased antibodies against PCV2 in colostrum to protect the piglets within the first days of life. At

this age, colostral antibody is the only specific immune mediator available to the piglets before they can generate their own.

This is the first evidence of a subclinical infection of PCV2 as determined by the daily weight increase of pigs during the fattening period. We consider weight gain of young animals as a mirror to reflect a combined effect of the virus on rapidly dividing cells necessary in the development of the immune-, hormonal- and nervous system influenced by the gastrointestinal or respiratory tract. The IgM antibodies specific to PCV2 not present in colostrum but determined in sera of unvaccinated piglets at 56 days of age indicate a perinatal infection even though viral DNA was not detected.

Therefore, in the absence of overt PCV2 associated diseases, small amounts of PCV2 might interfere with the maturation of newborn individuals possibly by interfering with rapidly diving cells. Circoviruses are extremely widespread, prone to genetic alterations and may adapt to different species [54]. The recent identification of circoviruses in stool samples of very young children should be considered in the light of our data. A similar spread as in other species should be monitored and immune responses analysed.

6. References

1. Harding J, Clark E: Recognizing and diagnosis postweaning multisystemic wasting syndrome. *Swine Health and Production* 1997, 5:201-203.
2. Allan G MB, Todd D, Kennedy S, McNeill F, Ellis J, Clark E.G, Harding J.C, Spun E, Bother A, Carrere C: Novel porcine circoviruses from pigs with wasting disease syndromes. *Vet Rec* 1998, 142:467-468.
3. Choi C, Chae C, Clark E: Porcine postweaning multisystemic wasting syndrome in Korean pig: detection of porcine circovirus 2 infection by immunohistochemistry

and polymerase chain reaction. *Journal of Veterinary Diagnostic Investigation* 2000, 12:151-153.

4. Hinrichs U, V.F. O, Pesch S, Wang L: Erster Nachweis einer Infektion mit dem porzinen Circovirus Typ 2 in Deutschland. *Tierärztliche Umschau* 1999, 54:255-258.
5. Kiupel M, Stevenson GW, Mittal SK, Clark EG, Haines DM: Circovirus-like viral associated disease in weaned pigs in Indiana. *Veterinary Pathology* 1998, 35:303-307.
6. LeCann P, Albina E, Madec F, Cariolet R, Jestin A: Piglet wasting disease. *Vet Rec* 1997, 141:660.
7. Segales J, Sitjar M, Domingo M, Dee S, Del Pozo M, Noval R, Sacristan C, De las Heras A, Ferro A, Latimer KS: First report of post-weaning multisystemic wasting syndrome in pigs in Spain. *Vet Rec* 1997, 141:600-601.
8. Allan G, Meehan B, Todd D, Kennedy S, F FM, Ellis J, Clark E, Harding J, Espuna E, Botner A, Charreyre C: Novel porcine circoviruses from pigs with wasting disease syndromes. *Vet Rec* 1998, 142:467-468.
9. Wiederkehr DD, Sydler T, Buergi E, Haessig M, Zimmermann D, Pospischil A, Brugnera E, Sidler X: A new emerging genotype subgroup within PCV-2b dominates the PMWS epizooty in Switzerland. *Vet Microbiol* 2009, 136:27-35.
10. Ellis J, Hassard L, Clark E, Harding J, Allan G, Willson P, Strokappe J, Martin K, McNeilly F, Meehan B, et al: Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. *Can Vet J* 1998, 39:44-51.
11. Allan GM, Ellis JA: Porcine circoviruses: a review. *J Vet Diagn Invest* 2000, 12:3-14.

12. Pallares FJ, Halbur PG, Opriessnig T, Sorden SD, Villar D, Janke BH, Yaeger MJ, Larson DJ, Schwartz KJ, Yoon KJ, Hoffman LJ: Porcine circovirus type 2 (PCV-2) coinfections in US field cases of postweaning multisystemic wasting syndrome (PMWS). *J Vet Diagn Invest* 2002, 14:515-519.

13. Segales J, Domingo M: Postweaning multisystemic wasting syndrome (PMWS) in pigs. A review. *Vet Q* 2002, 24:109-124.

14. Rosell C, Segales J, Plana-Duran J, Balasch M, Rodriguez-Arrioja GM, Kennedy S, Allan GM, McNeilly F, Latimer KS, Domingo M: Pathological, immunohistochemical, and in-situ hybridization studies of natural cases of postweaning multisystemic wasting syndrome (PMWS) in pigs. *Journal of Comparative Pathology* 1999, 120:59-78.

15. Seeliger FA, Brugmann ML, Kruger L, Greiser-Wilke I, Verspohl J, Segales J, Baumgartner W: Porcine circovirus type 2-associated cerebellar vasculitis in postweaning multisystemic wasting syndrome (PMWS)-affected pigs. *Vet Pathol* 2007, 44:621-634.

16. Allan GM, McNeilly F, Kennedy S, Daft B, Clarke EG, Ellis JA, Haines DM, Meehan BM, Adair BM: Isolation of porcine circovirus-like viruses from pigs with a wasting disease in the USA and Europe. *Journal of Veterinary Diagnostic Investigation* 1998, 10:3-10.

17. Steiner E, Balmelli C, Herrmann B, Summerfield A, McCullough K: Porcine circovirus type 2 displays pluripotency in cell targeting. *Virology* 2008, 378:311-322.

18. Yu S, Opriessnig T, Kitikoon P, Nilubol D, Halbur PG, Thacker E: Porcine circovirus type 2 (PCV2) distribution and replication in tissues and immune cells in early infected pigs. *Vet Immunol Immunopathol* 2007, 115:261-272.

19. McIntosh KA, Harding JC, Parker S, Ellis JA, Appleyard GD: Nested polymerase chain reaction detection and duration of porcine circovirus type 2 in semen with sperm morphological analysis from naturally infected boars. *J Vet Diagn Invest* 2006, 18:380-384.

20. Schmoll F, Lang C, Steinrigl AS, Schulze K, Kauffold J: Prevalence of PCV2 in Austrian and German boars and semen used for artificial insemination. *Theriogenology* 2008, 69:814-821.

21. Lopez-Soria S, Segales J, Rose N, Vinas MJ, Blanchard P, Madec F, Jestin A, Casal J, Domingo M: An exploratory study on risk factors for postweaning multisystemic wasting syndrome (PMWS) in Spain. *Prev Vet Med* 2005, 69:97-107.

22. West KH, Bystrom JM, Wojnarowicz C, Shantz N, Jacobson M, Allan GM, Haines DM, Clark EG, Krakowka S, McNeilly F, et al: Myocarditis and abortion associated with intrauterine infection of sows with porcine circovirus 2. *J Vet Diagn Invest* 1999, 11:530-532.

23. Kim J, Jung K, Chae C: Prevalence of porcine circovirus type 2 in aborted fetuses and stillborn piglets. *Vet Rec* 2004, 155:489-492.

24. O'Connor B, Gauvreau H, West K, Bogdan J, Ayroud M, Clark EG, Konoby C, Allan G, Ellis JA: Multiple porcine circovirus 2-associated abortions and reproductive failure in a multisite swine production unit. *Canadian Veterinary Journal-Revue Veterinaire Canadienne* 2001, 42:551-553.

25. Sanchez RE, Jr., Nauwynck HJ, McNeilly F, Allan GM, Pensaert MB: Porcine circovirus 2 infection in swine foetuses inoculated at different stages of gestation. *Vet Microbiol* 2001, 83:169-176.

26. Nielsen J, Ladekjaer-Mikkelsen AS, Bille-Hansen V, Lohse L, Botner A: PCV2-associated disease following intrauterine infection. *Proc18th Int Pig Vet Soc Congress, Hamburg* 2004, 1:14.

27. Park JS, Kim J, Ha Y, Jung K, Choi C, Lim JK, Kim SH, Chae C: Birth abnormalities in pregnant sows infected intranasally with porcine circovirus 2. *J Comp Pathol* 2005, 132:139-144.

28. Shen H, Wang C, Madson DM, Opriessnig T: High prevalence of porcine circovirus viremia in newborn piglets in five clinically normal swine breeding herds in North America. *Prev Vet Med* 2010, 97:228-236.

29. Borel N, Burgi E, Kiupel M, Stevenson GW, Mittal SK, Pospischil A, Sydler T: Three cases of "Postweaning Multisystemic Wasting Syndrome" (PMWS) due to porcine circovirus type 2 (PCV 2) in Switzerland. *Schweizer Archiv fuer Tierheilkunde* 2001, 143:249-255.

30. Welti S, Sydler T, Buergi E, Brugnera E, Sidler X: PDNS was not a homogenous enity in the Swiss PMWS epizooty from 2003-2006. *Proc 21th Int Pig Vet Soc Congress, Vancouver* 2010, 2:455.

31. Harding JC: The clinical expression and emergence of porcine circovirus 2. *Vet Microbiol* 2004, 98:131-135.

32. Tischer I, Bode L, Peters D, Pociuli S, Germann B: Distribution of antibodies to porcine circovirus in swine populations of different breeding farms. *Arch Virol* 1995, 140:737-743.

33. Magar R, Muller P, Larochelle R: Retrospective serological survey of antibodies to porcine circovirus type 1 and type 2. *Can J Vet Res* 2000, 64:184-186.

34. Opriessnig T, Yu S, Thacker E, Halbur PG: Derivation of porcine circovirus type 2-negative pigs from positive breeding herds. *J of Swine Health and Production* 2004, 12:186-191.

35. Staebler S, Buergi E, Litzenberger B, McCullough K, McNair I, McNeilly F, Pospischil A, Sydler T: Porcine circovirus as a possible cause of postweaning wasting in pigs in Switzerland. *Schweizer Archiv fuer Tierheilkunde* 2004, 146:461-468.

36. Harding J, Clark E, Ellis J: The clinical expression of porcine circovirus. In *Proceedings of the Allen D Leman Swine Conference Saint Paul, Minnesota*. 1999: 252-254.

37. Larochelle R, Magar R, D'Allaire S: Comparative serologic and virologic study of commercial swine herds with and without postweaning multisystemic wasting syndrome. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire* 2003, 67:114-120.

38. Sorden SD: Update on porcine circovirus and postweaning multisystemic wasting syndrome (PMWS). *Swine Health and Production* 2000, 8:133-136.

39. Madec F, Eveno E, Morvan P, Hamon L, Blanchard P, Cariolet R, Amenna N, Morvan H, Truong C, Mahe D, et al: Post-weaning multisystemic wasting syndrome (PMWS) in pigs in France: clinical observations from follow-up studies on affected farms. *Livestock Production Science* 2000, 63:223-233.

40. Madec F, Waddilove J: Control PCV2 or Control Other Factors? Several Approaches to a Complex Problem. In *Proceedings of the17th IPVS Congress;*

Ames, Iowa. Keynotes on the Merial Symposium and Brief Epidemiological Updates; 2002: 45-53.

41. Opriessnig T, Madson DM, Prickett JR, Kuhar D, Lunney JK, Elsener J, Halbur PG: Effect of porcine circovirus type 2 (PCV2) vaccination on porcine reproductive and respiratory syndrome virus (PRRSV) and PCV2 coinfection. *Vet Microbiol* 2008, 131:103-114.
42. Kixmöller M, Ritzmann M, Eddicks M, Saalmuller A, Elbers K, Fachinger V: Reduction of PMWS-associated clinical signs and co-infections by vaccination against PCV2. *Vaccine* 2008, 26:3443-3451.
43. Fort M, Sibila M, Perez-Martin E, Nofrarias M, Mateu E, Segales J: One dose of a porcine circovirus 2 (PCV2) sub-unit vaccine administered to 3-week-old conventional piglets elicits cell-mediated immunity and significantly reduces PCV2 viremia in an experimental model. *Vaccine* 2009, 27:4031-4037.
44. Chae C: A review of porcine circovirus 2-associated syndromes and diseases. *Vet J* 2005, 169:326-336.
45. Guillossou S, Lebon E, Mieli L, Bonnard M, Thomsen C, Thomsen C: Development of a quantification method to specific anti-ORF2 antibody using a blocking ELISA. In *Proceedings of the American Association of Swine Veterinarians Congress; San Diego, California*. 2008: 1-4.
46. McNeilly F, McNair I, Mackie DP, Meehan BM, Kennedy S, Moffett D, Ellis J, Krakowka S, Allan GM: Production, characterisation and applications of monoclonal antibodies to porcine circovirus 2. *Arch Virol* 2001, 146:909-922.

47. Staebler S, Sydler T, Buergi E, McCullough K, McNeilly F, Allan G, Pospischil A: PMWS: an emerging disease identified in archived porcine tissues. *Veterinary Journal* 2005, 170:132-134.

48. Opriessnig T, Patterson AR, Madson DM, Pal N, Ramamoorthy S, Meng XJ, Halbur PG: Comparison of the effectiveness of passive (dam) versus active (piglet) immunization against porcine circovirus type 2 (PCV2) and impact of passively derived PCV2 vaccine-induced immunity on vaccination. *Vet Microbiol* 2010, 142:177-183.

49. Butler JE, Zhao Y, Sinkora M, Wertz N, Kacskovics I: Immunoglobulins, antibody repertoire and B cell development. *Dev Comp Immunol* 2009, 33:321-333.

50. Desrosiers R, Clark E, Tremblay D, Polson D: Results obtained with a novel PCV2 Vaccine to protect multiple ages of pigs against PCVD. *Proc 5th Int Symp on emerging and re-emerging pig diseases (Krakow, Poland)* 2007:121.

51. Fachinger V, Bischoff R, Judicial SB, Saalmuller A, Elbers K: The effect of vaccination against porcine circovirus type 2 in pigs suffering from porcine respiratory disease complex. *Vaccine* 2008, 26:1488-1499.

52. Hansen MS, Haulage CK, Bille-Hansen V, Haggard S, DuPont K, Homedale P, Kinsman L, Larsen LE: Selection of method is crucial for the diagnosis of porcine circovirus type 2 associated reproductive failures. *Vet Microbiol* 2010, 144:203-209.

53. Bionomic FC, Bailer CA: Influence of nutritional deprivation on insulin-like growth factor I, somatotropin, and metabolic hormones in swine. *J Anim Sci* 1991, 69:755-760.

54. Li L, Kapoor A, Slikas B, Bamidele OS, Wang C, Shaukat S, Masroor MA, Wilson ML, Ndjango JB, Peeters M, et al: Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. *J Virol* 2010, 84:1674-1682.

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